

## Floral Biology and Pollination Mechanisms in Two *Viola* Species—From Nectar to Pollen Flowers?

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The genus *Viola* is represented by four related species in Brazil belonging to section *Leptidium*, one of the most primitive sections in the genus. Floral biology and pollination by bees were studied in *Viola cerasifolia* and *V. subdimidiata* in high-altitude areas in south-eastern Brazil. Flowers are zygomorphic and spurred. The five stamens are arranged in a cuff around the ovary, and pollen is released by means of apical connective projections, which form a cone surrounding the base of the style. The connective projections of the inferior stamens are elongated and curved to form a hook-shaped structure. Nectar-secreting tissue can occur in the basal connective appendages of the inferior stamens, which project into the spur. Flowers of *V. subdimidiata* secreted a mean volume of 0.14 µl nectar over a 24-h period; approx. 40 % of flowers did not secrete any nectar. The main pollinators of these *Viola* species are female bees belonging to the genus *Anthrenoides* (Andrenidae), which search mainly for pollen. These bees seem to be oligolectic and obtain large amounts of pollen from *Viola* by vibrating the flowers or by moving the hook repeatedly back and forth. Males of *Anthrenoides* patrol *Viola* clusters and also feed on nectar, acting as secondary pollinators. The basic floral structure in the genus *Viola* fits that of 'nectar flowers'. The uncommon hook-shaped projections, scanty nectar production, and behaviour of pollinators suggest that *V. cerasifolia* and *V. subdimidiata* are shifting their reward for pollinators from nectar to pollen. Based on floral morphology, this shift may be widespread in *Viola* sect. *Leptidium*.

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**Key words:** Andrenidae, *Anthrenoides*, buzz pollination, floral biology, melittophily, nectary, oligolectic bees, pollen flowers, *Viola*, Violaceae.

### INTRODUCTION

*Viola* is a large genus containing approx. 525–600 species. The genus is distributed mainly in the Northern hemisphere despite its probable Andean origin (Ballard *et al.*, 1999). The genus is well known for the presence of cleistogamous (CL) flowers and by the singularity of its chasmogamous (CH) flowers, which have attracted the attention of pollination biologists since Sprengel (1793). Floral biology and pollination mechanisms have been studied in several North American and European species (e.g. Knuth, 1904; Beattie, 1969a, b, 1971, 1972; Herrera, 1988), but we know of no published information regarding South American species.

There are only four species of *Viola* in Brazil; these belong to the predominantly austral section *Leptidium*. Species of this section occur mainly in the Andes, and constitute one of the two most primitive groups in the genus (Ballard *et al.*, 1999). In spite of considerable divergence in morphological traits, the four Brazilian species are closely related and apparently reflect a single evolutionary relict lineage, which is probably the most primitive within the section (H. E. Ballard, pers. comm.). These *Viola* species occur mainly in montane forests and grasslands in the south-eastern highlands of the country. These formations are

subtypes of the Brazilian Atlantic Forest, which is one of the most endangered ecosystems on earth (Mori *et al.*, 1981; Morellato and Haddad, 2000).

This paper reports observations on floral biology and pollination mechanisms of *Viola cerasifolia* A. St.-Hil. and *V. subdimidiata* A. St.-Hil. Based on floral morphology, nectar production, and the type and behaviour of pollinators, it is suggested that these *Viola* species are shifting their reward for pollinators from nectar to pollen, and are thereby moving away from the basal condition of 'nectar flower' which characterizes the family.

### MATERIALS AND METHODS

#### Study sites

The population of *Viola cerasifolia* studied was located in a high-altitude grassland (approx. 1600 m a.s.l.) at the Parque Nacional da Serra da Bocaina (PNSB; 22°44'S, 44°36'W), while that of *V. subdimidiata* was in a semi-deciduous broad-leaf montane forest at the Parque Nacional da Serra dos Órgãos (PNSO; 22°28'S, 43°02'W, approx. 1850 m a.s.l.), both in the Serra do Mar range, south-eastern Brazil. The vegetation of these sites has been described by Eiten (1970, 1992) and Safford (1999). These montane areas can be classified as Cfb, after Köppen climatic regions, i.e. mesothermic, with average annual temperatures of 12–20 °C, moderate winters and mild, wet summers

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(Safford, 1999). Annual rainfall ranges from 1500 to 2500 mm, with the rainy season occurring between October and March; less than 50 mm rain falls per month between June and August.

Both species are small perennial herbs that can reproduce clonally. *Viola cerasifolia* inhabits shaded, sheltered spots on grassy fields, mainly in slits of exposed granite rock. It grows in small clusters and only approx. 40 individuals of this species were found at Serra da Bocaina. *Viola subdimidiata* occurs in large clusters along partially shaded forest edges. It was estimated that there were around 1000 individuals in the study population at Serra dos Órgãos. *Viola cerasifolia* flowered from December to February at Serra da Bocaina (from 1998 to 2000). The flowering phenology of *V. subdimidiata* was not recorded but data from herbarium collections indicate a similar flowering pattern to that of *V. cerasifolia*. Cleistogamous flowers were detected in *V. subdimidiata* but not in *V. cerasifolia*. However, it is expected that CL flowers also occur in the latter species. Voucher specimens of both species were deposited in the Herbarium of the Universidade Estadual de Campinas (UEC).

#### *Floral biology and pollinator observations*

A total of 21 and 12 h was spent recording floral visitors to *V. cerasifolia* during the 1999–2000 flowering season and to *V. subdimidiata* in January 2002. The frequency of each insect species was recorded, as was their behaviour when searching for floral resources. To check whether visitors looked for nectar, a small piece of the corolla spur was cut in some flowers during the field observations. Stigma receptivity was tested by the H<sub>2</sub>O<sub>2</sub>-catalase activity method (Zeisler, 1938). Pollen viability of *V. cerasifolia* was estimated by cytoplasmic staining, using the aceto-carmin technique (Radford *et al.*, 1974).

#### *Floral morphology*

For scanning electron microscopy (SEM), 12 flowers of *V. cerasifolia* were fixed in 2.5 % glutaraldehyde in 0.05 M sodium cacodylate buffer, pH 7.0, before being dehydrated in a graded ethanol/acetone series. Flowers were critical point dried in a Balzers CPD 030 instrument (Balzers, Liechtenstein) using CO<sub>2</sub> as the replacement fluid. Dried specimens were mounted on stubs and coated with gold in a Balzers SCD 050 sputter coater. Material was examined using a Phillips 505 (Eindhoven, The Netherlands) SEM at 25 kV.

#### *Nectar secretion*

Flowers of *V. cerasifolia* and *V. subdimidiata* do not produce nectar or else secrete it in very small quantities; any nectar secreted forms a thin layer covering the tips of the staminal appendages that are projected into the corolla spur. Thus, direct nectar measurements are not possible. To determine the volume of nectar secreted by *V. subdimidiata*, 70 flowers selected at random were tagged and bagged (using mosquito netting) at the beginning of anthesis. The

following morning the corolla spur was carefully removed and nectar was extracted using small rectangular strips of chromatography paper (Whatman No. 1; Maidstone, UK). The upper level reached by the nectar was marked on the paper strips. Nectar volume was estimated by measuring the height of the mark on the paper with the assistance of a digital calliper rule. The flowers used for nectar measurements were fixed in FAA. To investigate a possible relationship between the number of nectary stomata and nectar volume, 15 nectar-secreting and 15 non-secreting flowers of *V. subdimidiata* were chosen. Distal parts of the staminal appendages were cleared using NaOH (10 % aqueous solution), washed in ethyl alcohol : water (3 : 1) and stained with Lugol solution. The number of stomata was counted under a light microscope (Johansen, 1940). The same procedure was applied to six flowers of *V. cerasifolia*.

## RESULTS

#### *Floral biology*

Flowers of *V. cerasifolia* (Fig. 1A) and *V. subdimidiata* (Fig. 1C) are indistinguishable, so the following description of floral morphology applies to both species. Flowers are horizontal at the start of anthesis, but the pedicel elongates during and after anthesis, changing the position of the flower so that young fruits are placed on the ground. Flower morphology generally follows the description given by Beattie (1969a, 1971) for three British *Viola* species. The zygomorphic, pentamerous and spurred flowers are approx. 15 × 12 mm in diameter. The corolla tube is 3–4 mm long and the corolla opening is 2–3 mm in diameter. Petals are smooth and lack the tufts of hairs that are common in other species of the genus. The spur of the inferior petal is short, approx. 1–2 mm in length. The main colour of the petals is violet, with distal parts being pale violet or white, and with dark violet ribs forming nectar guides. The base of the inferior petal is vivid yellow and white, providing a contrasting pattern (Fig. 1A). During anthesis, petals gradually lose their colour, becoming completely white or pale lilac by the end of anthesis. A sweet fragrance was detected, especially during the morning hours. Each flower lasts about 6 d.

The androecium is the most elaborate floral part in *V. cerasifolia* (Fig. 2A) and *V. subdimidiata*. The five stamens have short, broad filaments. Anthers are introrse and fused longitudinally through many papillae (Fig. 2A), in such a way that they form a cuff around the ovary. The basal parts of the two inferior anthers have connective appendages approx. 1 mm long (Fig. 2A) projecting into the corolla spur. Connectives also have projections in their distal part. The projections of the superior and lateral stamens (1–2 mm long) form a hollow cone around the style (Figs 2A and 3A). The projections of the inferior stamens are joined to the lateral ones through papillae in their proximal part. The inferior projections are especially elongate, ending in a hook-shaped tip (approx. 1 mm long), hereafter called the 'hook' (Figs 2A and 3A). Each anther (approx. 2.5 mm) opens longitudinally; however, the fusion between anthers leads to the pollen being presented at the inferior part of the

cone. Dehiscence of the anther valves begins at the distal end; the suture then opens up, like a zip, down to the anther's base (see Beattie, 1969b). Dehiscence of anthers follows a superior–lateral–inferior sequence, thus pollen is progressively available to pollinators throughout the period of anthesis. The 3-aperturate pollen grains are spheroid and small (approx. 20  $\mu\text{m}$  in diameter), with a smooth and non-reticulate exine. The pollen viability of *V. cerasifolia* ranged from 66.1 to 79.6 % (mean 72.5 %,  $n = 5$ ).

The gynoecium is syncarpous, superior and three-carpelled, with one unilocular ovary and a mean ( $\pm$  s.d.) number of ovules of 26.0 ( $\pm$  2.67,  $n = 10$ ) and 23.7 ( $\pm$  1.89,  $n = 10$ ), respectively, for *V. cerasifolia* and *V. subdimidiata*. The 2–3-mm-long style is tubular and soft without any constricted area of flexure, in contrast to that of other species studied (see Beattie, 1969a, b). It contains a lumen, filled by a mucilaginous substance, that is continuous with the cavity of the ovary. The stigma is simple and truncate, without lips (Fig. 2A), and its border is moistened by the mucilage. Following pollen grain deposition the stigma closes (Fig. 3B). The time course of stigma closing appears to be highly variable among flowers in both species.

#### Nectar and nectaries

Nectar is produced in the distal parts of the basal connective appendages (hereafter called 'nectary appendages'; Fig. 2A) by a mesenchymal nectary, and is exuded by modified stomata (Fig. 2B; see Vogel, 1998). Nectar production was very low and only a thin layer of secretion covering the tips of the nectary appendages was detected. Forty-three flowers of *V. subdimidiata* secreted between 0.03 and 0.45  $\mu\text{l}$  of nectar over a 24-h period. A further 27 flowers (38.6 %) did not secrete nectar. The mean ( $\pm$  s.d.) nectar volume was 0.14  $\mu\text{l}$  ( $\pm$  0.14,  $n = 70$ ), or 0.22  $\mu\text{l}$  ( $\pm$  0.12) if only the 43 secreting flowers are considered. Data on nectar volume are not available for *V. cerasifolia*, but 16 out of 20 flowers studied in the field secreted no nectar. Furthermore, nectary appendages of some non-secreting flowers were vestigial (Fig. 2C).

The mean ( $\pm$  s.d.) number of nectary stomata per flower (i.e. considering the two nectary appendages) was 72.9 ( $\pm$  28.99,  $n = 15$ ) and 61.1 ( $\pm$  19.52,  $n = 15$ ) for, respectively, nectar-secreting and non-secreting flowers of *V. subdimidiata*. The number of stomata did not differ significantly between these two groups of flowers ( $t = 1.20$ , d.f. = 14,  $P = 0.25$ ). Furthermore, the Pearson correlation between number of stomata and nectar volume was low ( $r = 0.465$ ,  $n = 15$ ). Two-thirds of *V. cerasifolia* flowers ( $n = 18$ ) lacked stomata on their nectary appendages. Those

flowers that did possess stomata had about 30. Thus the number of nectary stomata does not seem to be related to nectar secretion in *V. subdimidiata*, but may be related in *V. cerasifolia*.



FIG. 1. Flower and pollinators of *Viola cerasifolia* and *V. subdimidiata* in south-eastern Brazil. A, Flower of *V. cerasifolia* in front view; note the conspicuous yellow patch at the entrance to the corolla tube. B, A female of *Anthrenoides* sp. (Andrenidae) visiting a flower of *V. cerasifolia* in the supine position. In this position the bee's body touches the stigma. Note the yellow pollen mass on the bee's hind leg. C, A male of *Anthrenoides* sp. visiting a flower of *V. subdimidiata* in search of nectar.

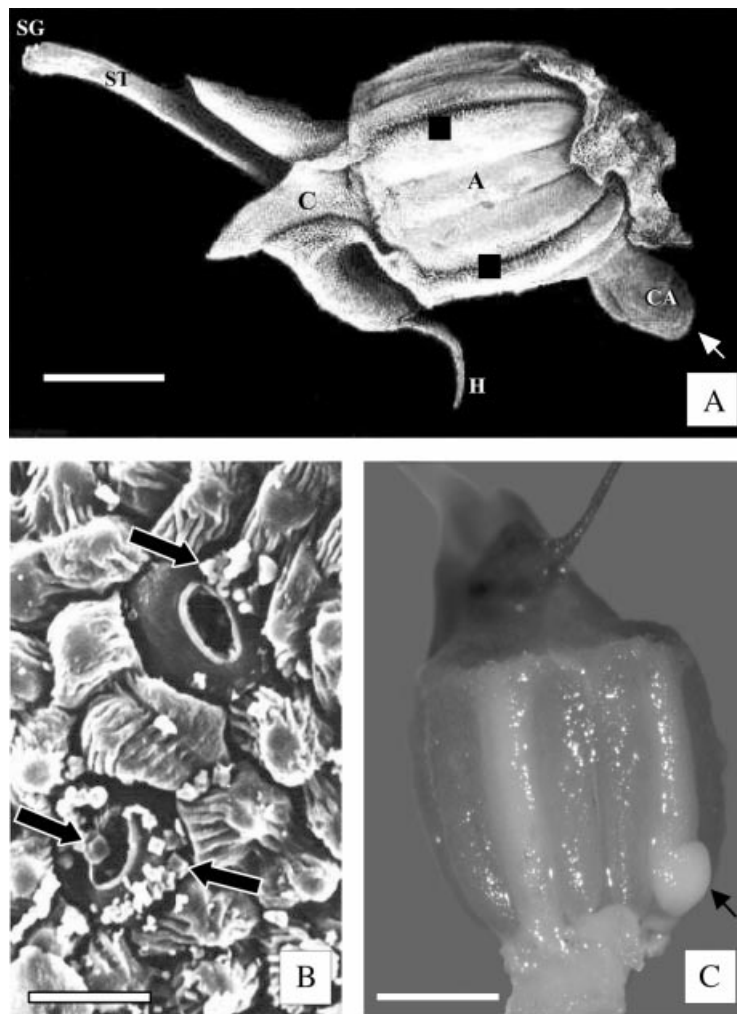


FIG. 2. Floral structure of *V. cerasifolia*. A, SEM of the gynoecium and androecium in longitudinal view. During preparation the hook was moved in the direction of the connective appendages, thus opening the cone around the style. The same movement is carried out by bees when they enter the corolla tube to collect pollen, and in this way pollen is released. Note the region of anthers joined by papillae (squares). Nectar is produced in the tip of the basal connective appendage ('nectary appendage') (arrow). Bar = 1 mm. A, Anther; C, cone; CA, connective appendage; H, hook; SG, stigma; ST, style. B, SEM of the nectary appendage tip showing modified stomata through which nectar is exuded. Crystals on the surface of the stomata (arrows) seem to be sugar, but may be preparation artefacts. Bar = 17  $\mu$ m. C, Detail of the inferior stamens showing a vestigial nectary appendage (arrow). Note the absence of nectary appendage on the anther on the left-hand side. Bar = 0.8 mm.

#### Pollination mechanisms and pollinators

Mechanisms and agents of pollination were similar for *V. cerasifolia* and *V. subdimidiata*, which were pollinated exclusively by small solitary bees. In all visits, bees adopted a supine feeding posture (Fig. 1B and C). To achieve that position, bees landed directly on the superior petals with their heads oriented to the inferior petal, or rotated their bodies 180° after landing on the inferior petal. The inferior petal has a curvature forming a chamber at the beginning of the corolla tube. To put their head into the tube chamber, bees aligned their bodies in a perpendicular position in relation to the main axis of the corolla tube (Fig. 1B). In such a position, the ventral parts of the bee's body touched the stigma just before it fully entered the tube to collect pollen or probe for nectar. The contact with the stigma at the beginning of the bee's visit could favour deposition of

outcrossed pollen. Inside the tube, bees used their forelegs to hold onto the stamen cuff. When a bee entered the floral tube, its head pushed the hook forward in the direction of the corolla base. As the inferior projections are connected to the lateral ones, this hook displacement moved them down (Fig. 2A). As a result, a small amount of pollen—already within the cone—was released over the bee's ventral parts.

The most important pollinators were bees—especially females—of the genus *Anthrenoides* (Panurginae, Andrenidae). The taxonomy of this bee group is unclear, and it is possible that the *Anthrenoides* bees pollinating each species of *Viola* belong to either the same species or to two very similar species. In any case, no differences in behaviour of these bees were observed during visits to both *Viola* species. Pollen is the chief resource offered, and females of *Anthrenoides* were observed collecting pollen on all of their visits. The movement of the hook when the bee

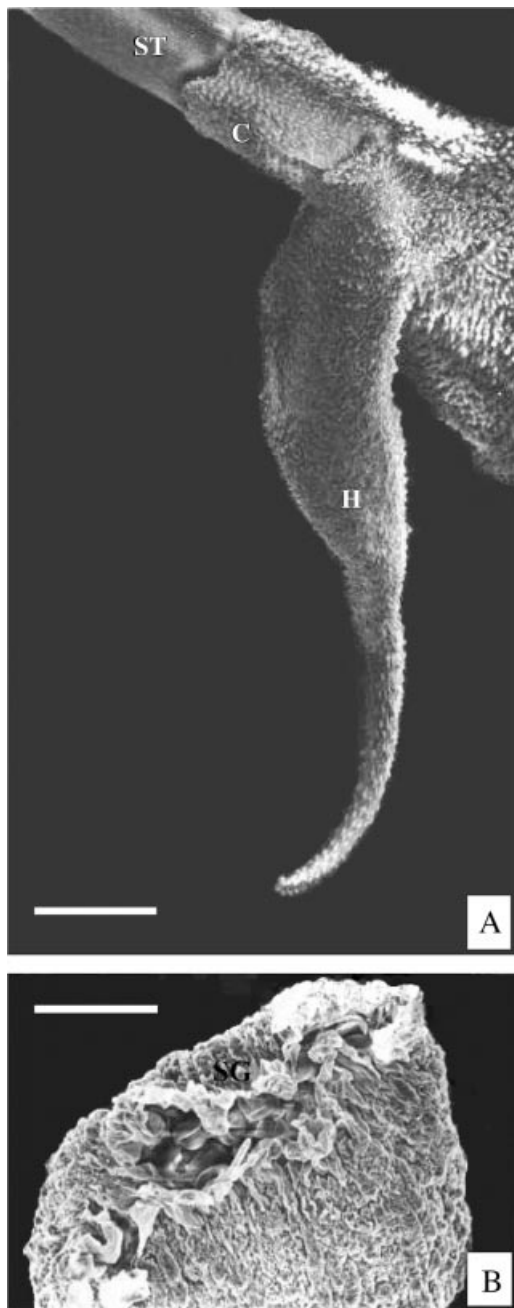


FIG. 3. SEM of parts of a *V. cerasifolia* flower. A, Detail of the hook. Note the cone close to the style (resting position). Bar = 0.3 mm. B, The stigmatic cavity is closed after pollen deposition. Bar = 60  $\mu$ m. C, Cone; H, hook; SG, stigma; ST, style.

enters the floral tube is the primary mechanism of pollen gathering. Two features of the behaviour of *Anthrenoides* bees improved pollen collection: vibration (buzz-pollination) and 'backward-forward movements'. The females harvested pollen by vibration while holding onto the anther cuff. Furthermore, these bees were seen collecting pollen by retracting their bodies from the corolla tube until only the head remained inside (Fig. 1B), and then moving forward to enter the tube again. This backward-forward movement was

repeated two to four times at each flower visited. As a result, the cone was opened several times and a larger amount of pollen was released. Each individual of *Anthrenoides* was observed to collect pollen either by vibration or by backward-forward movements each time it foraged.

Male *Anthrenoides* bees were commonly seen at *Viola* patches searching for females. They alternated between patrolling flights and stopovers on leaves and flowers of *Viola* or on surrounding stones or plants of other species. Some motionless males were also found inside *Viola* flowers, mainly in those at the end of anthesis. Patrolling bees flew towards a flower, hovered very fast in front of it, and then moved to another flower. At times they interrupted the patrols to visit the flowers (Fig. 1C). During the visits, male bees entered the flower in a similar way to the females (in a supine position), but with their proboscis extended, indicating that they were in search of nectar. A few pollen grains were deposited on the ventral surface of their bodies, so they may pollinate the flowers. During visits to flowers in which the corolla spur was removed, males directed their glossa to the tips of the connective appendages, making it clear that their search was for nectar. Thus, nectar seems to be a secondary resource, used by males and perhaps also by females. The foraging pattern differed markedly between males and females of *Anthrenoides*, since males frequently stayed at the same patch for long periods, whereas females characteristically visited flowers of many clusters on each foraging trip.

Bees belonging to the genera *Augochlorodes* and *Dialictus* (Halictidae) and to an unidentified genus of Andrenidae also visited *Viola* flowers, the last being observed only in *V. subdimidiata*. These bees visited flowers in search of pollen, adopting a supine posture as they entered the corolla tube. The bees may pollinate *Viola* flowers, but they can be only minor pollinators due to the scarcity of their visits. Other visitors were syrphids, which landed on the inferior petal and fed directly on pollen grains deposited on this petal; they did not touch the stigma or enter the corolla tube. *Plebeia* cf. *saiqui* (Friesi) (Meliponini, Apidae) also visited flowers of *V. subdimidiata*. Many individuals of this bee species were seen actively collecting pollen from two species of *Borreria* (Rubiaceae) near a *Viola* cluster, and some individuals occasionally visited the latter in search of pollen. They did not effect pollination due to incorrect body positioning. They were also unable to move the hook to release pollen, and damaged the anthers in the process of pollen collection.

## DISCUSSION

The manifest zygomorphic and spurred flowers of *Viola* species support the classical view that they are 'nectar flowers' pollinated by bees (Knuth, 1904; Lovell, 1918). Beattie (1971, 1972) showed that nectar is the main floral resource in three *Viola* species pollinated by hoverflies, bee flies and butterflies, in addition to large and small bees. However, some of these pollinators also utilize pollen as a food source, characterizing a more generalist pollination system in these *Viola* species (Beattie, 1971, 1972). In contrast to other species in the genus, pollen is the main

floral resource of *V. cerasifolia* and *V. subdimidiata*. This conclusion is supported by three observations: (1) scanty nectar production and the reduced size of both the corolla spur and nectary appendages (including the occurrence of vestigial appendages); (2) the shape and size of the inferior connective projections (hook), a structure apparently related to pollen collection by bees; and (3) the behaviour of *Anthrenoides* female bees, collecting pollen on all visits either by vibration or by backward–forward movements.

The pollinators of *V. cerasifolia* and *V. subdimidiata* visit the flowers exclusively in the supine position (sternotriby). Beattie (1974) argued that sternotriby is the primitive condition in the genus, and that species belonging to more recent sections would show a progressive decrease in sternotriby. However, the infrageneric classifications used by Beattie to propose this scenario (Clausen, 1927, 1929; Gershoy, 1928) are not completely in accordance with a recent phylogenetic study based on DNA sequences (Ballard et al., 1999). In both *Viola* species studied here, sternotriby seems to be related to floral structures adapted to pollen collection by bees. Furthermore, these floral traits may be derived characters in the section *Leptidium* (S. Vogel, pers. comm.).

Vibration (buzz-pollination) is typically observed in ‘pollen flowers’ with poricidal anthers, such as found in many Solanaceae and Melastomataceae species (Buchmann, 1983). Although anthers of *V. cerasifolia* and *V. subdimidiata* dehisce longitudinally, they function as a single poricidal anther due to the intimate contact of anthers and the arrangement of the connective projections. This elaboration for pollen presentation is analogous to that described in other taxa with longitudinal anthers functioning as poricidal ones, as in *Chamaecrista* species (Gottsberger and Silberbauer-Gottsberger, 1988) and in some genera of Ochnaceae (Kubitzki and Amaral, 1991) and Epacridaceae (Houston and Ladd, 2002). The gynoeceum/androecium arrangement in *V. subdimidiata* and *V. cerasifolia* (Fig. 2A) resembles, to some extent, that of ‘*Solanum*-type flowers’ (after Vogel, 1978), which are characteristic of buzz-pollination and are usually associated with small, dry pollen grains and the absence of nectar (Buchmann, 1983). Similarities between these *Viola* species and *Solanum*-type flowers apparently represent an example of morphological convergence, and in this sense the floral traits of *V. cerasifolia* and *V. subdimidiata* could be, at least in part, a result of evolutionary pressures exerted by pollen-collecting bees that are able to pollinate by vibration (see also Kubitzki and Amaral, 1991; Houston and Ladd, 2002).

Female *Anthrenoides* bees gathered pollen from *Viola* species by means of backward–forward movements, a hitherto unreported means of pollen collection. Of the 124 plant species surveyed as part of a study of pollination biology at the community level in the high-altitude grasslands of Serra da Bocaina (Freitas, 2002), these bees were observed only in the flowers of *V. cerasifolia*. Oligolectic bees restrict pollen collection to a few related plants (Linsley, 1958) and frequently show behavioural, morphological or physiological traits associated with the gathering and transport of pollen of certain flowers (Gaglianone, 2000). In this sense, the fact that *Anthrenoides* bees visit

only *V. cerasifolia* flowers in the community, their behaviour in pollen collection and the presence of males patrolling *Viola* clusters indicate that these bees could be oligolectic foragers in the grasslands of Serra da Bocaina. Data on *V. subdimidiata* from Serra dos Órgãos, a site located more than 100 km away from Serra da Bocaina, reinforce this proposal. Furthermore, nectar production by some of the flowers of these species of *Viola* could be directly related to the reproductive success of *Anthrenoides* rather than to flower pollination, since males randomly visit the flowers and receive low pollen loads. The male strategy of establishing mating territories at feeding sites of the females (i.e. *Viola* plants) could be stimulated by the presence of nectar, which the males consume. Possible reproductive increments for the bees at the populational level may reflect on the reproductive success of the plant if the interaction between *Anthrenoides* bees and these *Viola* species is highly specific. Several bee species belonging to the Panurginae in South America exhibit oligolectic foraging behaviour, such as *Callonychium petuniae* (Wittmann et al., 1990) and *Cephalurgus anomalus* (Gaglianone, 2000), and further studies may clarify the degree of specificity in the interaction between species of *Viola* sect. *Leptidium* and species of *Anthrenoides*.

Some of the floral traits of *V. cerasifolia* and *V. subdimidiata* seem to be plesiomorphic for the genus, such as the simple and truncate stigma, and the absence of lateral hairs on the corolla. In contrast, the hook-shaped anther projection, the reduction of both corolla spur and nectary appendages, and the scanty nectar secretion are apparently derived traits, which would reflect adaptations for pollination by pollen-collecting bees. Furthermore, the occurrence of nectar is plesiomorphic in the family since it appears in more primitive members with actinomorphic flowers, such as *Rinorea* (Vogel, 1998). All species of *Viola* sect. *Leptidium* except for *V. arguta*, which has ornithophilous flowers, have highly reduced spurs relative to the more basal *Viola* sect. *Rubellium* and other Latin American groups of *Viola* (H. E. Ballard, pers. comm.). Thus, the reduced spur in *Viola* sect. *Leptidium* as a whole is probably apomorphic. Flowers of a Brazilian species (*V. gracillima*) have been examined; the floral structure was found to be similar to that of *V. cerasifolia* and *V. subdimidiata*, with a short spur and prolonged staminal projections (hook). Furthermore, S. Vogel (pers. comm.) has failed to find nectar in other species of this section, such as *V. stipularis* from Colombia, *V. sumatrana* from Borneo and *V. hederacea* from Australia (the latter studied in cultivation). These observations indicate that ‘pollen flowers’ may be widespread in this genus section, an idea first suggested by S. Vogel (pers. comm.). Based on floral traits and the behaviour of pollinators, flowers of *V. cerasifolia* and *V. subdimidiata*, as well as those of other species of *Viola* sect. *Leptidium*, seem to have evolved towards ‘pollen flowers’ from the primitive state in the genus of ‘nectar flowers’.

The means by which natural selection might favour pollen instead nectar as a floral reward is an intriguing question, since pollen is the vehicle for gametes, and also the pollen supply of flowers is strictly limited (for a



interesting view in this regard, see Westerkamp, 1996). Thus, it is expected that the availability of pollen as a reward for bees would be limited so that as much pollen as possible is available to effect fertilization, improving the male reproductive success (Harder and Thomson, 1989; Westerkamp, 1996). However, pollen is recognized as a floral reward in several taxa, especially for *Solanum*-type flowers. It is therefore reasonable to expect that under certain circumstances a plant might suppress nectar secretion in favour of extra pollen production to feed an insect, if it can both attract more efficient pollinators and discourage some inconstant visitors, which may, for example, lose many pollen grains during interspecific flights (see Waser, 1978). A possible scenario to promote pollen as a reward could involve: (1) floral structures and mechanisms that restrict access to pollen, which is then available to only a few bee species with particular morphology and/or behaviour; (2) the bees capable of pollen collection are oligolectic, which promotes flower fidelity; and (3) the bees offered this floral reward are solitary, and populations are small, thus reducing the competition for pollen in relation, for example, to social bees with large colonies. In a broad sense, these characteristics are typical of some very specialized plant–pollinator interactions involving bees and pollen flowers, as described by Wittmann *et al.* (1990) and Stehmann and Semir (2001), as well as for *V. cerasifolia* and *V. subdimidiata* and their pollinators. To test directly such an evolutionary scenario is not an easy task, but studies involving other species of *Viola* sect. *Leptidium* and species of related sections could be illuminating.

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